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(54) Title: THERAPEUTIC METHODS FOR TREATING SUBJECTS WITH A RECOMBINANT ERYTHROPOIETIN HAVING HIGH ACTIVITY AND REDUCED SIDE EFFECTS

(57) Abstract: The invention discloses several novel therapeutic properties and methods of treatment using the recombinant erythropoietin prepared by expression from the Apa I restriction fragment of human genomic erythropoietin DNA transformed into baby hamster kidney cells (BHK) according to U.S. Patent No. 5,688,697 to Powell. This recombinant erythropoietin designated herein as Epoetin Omega is shown to possess several unexpected and superior qualities over other recombinant erythropoietins such as those designated Epoetin Alfa and Beta which are prepared from genomic or cDNA expressed in Chinese Hamster Ovary (CHO) according to U.S. Patent Nos. 4,703,008 and 5,955,422 to Lin. The superior properties of Epoetin Omega include, but are not limited to, a much higher potency, a much more rapid response (i.e. no latency), longer effective serum levels, much lower antigenicity in human subjects, therapeutic activity in subjects non-responsive to the other epoetins, fewer adverse side effects such as incidents of thrombosis, reduced nausea, reduced pain at the site of injection, reduction in body pain, and most significantly, the absence of, or reduced risk of, increased blood pressure or hypertension. These novel properties provide for novel therapeutic methods including, treatment of anemia and treatment of conditions other than anemia such as fatigue or vascular pain, treatment in patients adversely effected by hypertension such as patients with heart conditions or at increased risk of thrombosis, treatment in oncology settings with and without chemotherapy or radiation therapy, and treatment with novel dosing regimens that include much lower doses and lower administration frequencies of as few as once per week or less.

resulting in glycoproteins with differing biological activities. In the case of Epoetin Omega, broad peak fractions selected from a final isoelectric purification step, *in vivo* assay results using a polycythemic mouse assay typically show a range from about 40,000 to about 65,000 IU/mg. More narrowly selected peak fractions have an *in vivo* activity in the range of 90,000 IU to 120,000 IU per mg. For Epoetin Alfa, *in vivo* activity of pharmaceutical preparations typically are in the range of about 110,000 IU per mg. Pharmaceutical preparations are tested in a quality assurance / quality control process using the polycythemic mouse assay before being released for human use. 191:1069-1087), values ranging from about are observed for Epoetin Omega. Radioimmunoassay results indicate an *in vitro* biological activity in the range of about 200,000 to about 240,000 U/mg for Epoetin Omega. Purified urinary EPO has been reported to have an *in vivo* activity from about 45,000 IU upwards to about 75,000 or more per mg. In addition, there are likely corresponding differences in the secondary or tertiary structures of the recombinant Erythropoietins (i.e., protein structure/folding) as well as the established differences in carbohydrate composition and bonding strength thereof, as well as stability of the various glycoproteins even though the primary protein sequence may be identical. Each known form of recombinant erythropoietin is a glycoprotein having a myriad of complex carbohydrate chains that include sugars that are N-linked to amino residues and/or O-linked to hydroxy residues. However, the content amount, number, position, bond strength, structure and composition of the carbohydrate linkages differ between the different recombinant erythropoietins and between urinary human erythropoietin. The structure and composition of Epoetin Omega carbohydrate residues has been described for example, by Nimtz *et al.* *Eur. J. Biochem.* 213:39, (1993); Tsuda *et al.* *Eur. J. Biochem.* 188:405, (1990); and Sytkowski *et al.*, *Biochem. Biophys. Res. Comm.* 176:698, (1988) each of which are incorporated herein by reference in their entirety.

Sytkowski, *et al.*, reports the results of sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the Epoetin Omega, which estimates that the glycoprotein has an average molecular weight (ca. 35 kDa) which is comparable to that found for urinary human erythropoietin glycoprotein (34-39 kDa; *see, e.g.*, Miyake, T. *et al.*, in *J. Biol. Chem.* (1977) 252:5558-5564). Additional studies under isoelectric focusing conditions show that Epoetin Omega is comprised of multiple isoforms (i.e., by IEF, about 6-8 isoforms in broad cut fractions and about 6 isoforms in peak fractions) which indicate differing types and amounts of

For treatment of common dialysis anemia, the average titration dose for Epoetin Alfa is typically about in the range of about 150-450 IU/kg per week divided into three doses per week, with an average of about 200 IU/kg or more per dose. Similarly, the typical maintenance dose for Epoetin Alfa is 225 IU/Kg per week divided into two or three doses, with 25% of patients requiring more than 600 IU/Kg per week. In contrast the average titration dose for Epoetin Omega invention is about 32 IU/kg, two to three times per week and the average maintenance dose is 23 IU/kg two to three times, or 40 to 100 IU/kg per week divided into one, two or three times per week; and the average Epoetin Omega maintenance dialysis dose is 20 to 70 IU/kg per week divided into one, two or three doses per week. This is illustrated, for example, in Figure 8, which shows high, low, and average dose requirements for Epoetin Alfa in comparison to Epoetin Omega for treatment of anemia during the maintenance period for dialysis patients. A typical maintenance dose for Epoetin Omega applied after a target hemoglobin value has been reached is about 1/2 to about 1/3 the amount used during the titration phase. The dose can be reduced to a lesser dose frequency than with Epoetin Alfa or Beta, in part because of the longer bioavailability and increased potency of Epoetin Omega. An increase in a dose during the maintenance phase is seldom needed for Epoetin Omega. The doses should be adjusted in small amounts, typically by about 5 to about 25 IU/kg per week. A typical weekly Epoetin Omega dose for 60-70% of hemodialysis patients was about 40 to about 60 IU/Kg/week. Approximately 45% of the patients could maintain a target hemoglobin level without any drug at all for one or two or even three weeks. Therefore, with Epoetin Omega once weekly injections of about 50-150 or 40-100 IU/kg can also be used for a large number of patients. Since aversion to injections/needles is a normal condition for dialysis or chronic disease patients, this lower frequency of actual administration is a big advantage, especially if the dose must be given s.c. route (more painful because of needles and nerves in the skin) to achieve yet a lower total weekly dose. This differs substantially from any known treatment with Epoetin Alfa or Beta where dose frequency of once a week is not effective so that even at doses as high as 200 IU/kg are not sufficient to maintain hemoglobin levels in the target range. The use of much lower doses of Epoetin Omega and less frequency of injection/administration results in several concomitant advantages, including lower total cost of therapy, reduced risk of EPO dose related side effects, and less to no likelihood of "creeping" dosage requirements than are exhibited by patients treated with Epoetin Alfa or Beta.

off after day 4 till the painful condition returned by day 8. On 12 or more occasions, this person has self administered Epoetin Omega at single dose of 4,000 IU with repeating successful results of significant or elimination of body/muscle/tissue pain. In addition, this patient reports similar improvements in "mood" or "sense of well being". Further, patient has been borderline hypertensive for 20 years with a lower blood systolic pressure ranging from 95 to 105. Following administration of Epoetin Omega, there was no increase in blood pressure, which is monitored daily. This report is consistent with that of the patient in Example 3 who reported on initial use, that she thought she was given a "pain shot" by "mistake" as her body pain from the chronic disease and cancer had subsided within minutes of administration of Epoetin Omega. In that case she has continued over months of treatment with Epoetin Omega to report routine reduction or elimination of body pain upon the administration of Epoetin Omega.

Surprisingly, it was also found that erythropoietins, in particular Epoetin Omega and related forms, as described above and claimed in the following claims, are suited to treat and/or prevent typical forms of jet lag, such as they occur after e.g. transatlantic or transpacific flights. Symptoms of jet lag or fatigue, tiredness, lack of concentration, and other disorders of the autonomous nervous system related to jet lag (dysrhythmia). Normally, the symptoms of jet lag last for 3 to 7 days before the organism has adapted to the different time zone it is confronted with.

Administration of typically 25 to 30 IU of erythropoietin per kg body weight in advance to or after occurrence of symptoms of jet lag suppresses or largely deletes such symptoms. Administration within 48 h after arrival at the destination is best. Normally, one to three administrations at an interval of 24 to 72 h are sufficient. Of course, erythropoietins are also active against other forms of dysrhythmia.

Yet another field of activity of erythropoietins, in particular Epoetin Omega and its related forms, is in the treatment of chronic heart conditions, such as chronic heart failure or heart insufficiency. Surprisingly, Epoetin Omega has shown a much higher activity and less side effects than Epoetin Alpha.

Erythropoietins have been widely used in the treatment of renal failure patients. Renal failure is often accompanied by heart failure, and is one of the leading causes of death for such

32. The use of claim 29 or 30, wherein the adverse side effect is selected from the group consisting of increased blood pressure, hypertension, thrombosis and increased platelet count.

33. The use of claim 29 or 30, wherein the condition is selected from the group consisting of hypertension, thrombosis, a heart condition, cancer, an autoimmune disease, liver dysfunction, hepatitis and treatment by chemotherapy or radiation therapy.

34. The use of claim 29 or 30, wherein the therapeutic benefit is selected from the group consisting of increased RBC, increased HCT, increased hemoglobin, increased vigor, increased mental acuity or decreased pain.

35. The use of claim 29 or 30, wherein the recombinant erythropoietin is administered at a dose of 5-150 IU/Kg, one to three times per week.

36. The use of claim 35, wherein the recombinant erythropoietin is administered at a dose of 75-200 IU/Kg, once per week.

37. The use of claim 29 or 30, wherein the symptom is associated with a cancer therapy.

38. The use of claim 37, wherein the cancer therapy is a chemotherapy.

39. The use of claim 38, wherein the chemotherapy is a cisplatin therapy.

40. The use of claim 38, wherein the erythropoietin is administered before, during or after the cancer therapy.

41. The use of claim 37, wherein the cancer therapy is a radiation therapy.

42. The use of claim 1, wherein the chronic heart failure is associated with renal failure and/or diabetic condition of a patient.

43. The use of claim 1, wherein the chronic heart failure is associated with cancer and/or cancer therapy.